Available online at www.sciencedirect.com
ScienceDirect
Journal of Hazardous Materials 145 (2007) 256–262
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

A kinetic study of biological Cr(VI) reduction in trickling filters with different filter media types

E. Dermou, D.V. Vayenas *

Department of Environmental and Natural Resources Management, University of Ioannina, Seferi 2, 30100 Agrinio, Greece

Received 17 June 2006; received in revised form 8 November 2006; accepted 12 November 2006
Available online 17 November 2006

Abstract

Two pilot-scale trickling filters were used in order to estimate Cr(VI) reduction through biological mechanisms in biofilm reactors operated in SBR mode with recirculation using different filter media types, i.e. plastic media and calcitic gravel. The feed concentrations of Cr(VI) examined were about 5, 10, 20, 30, 50 and 100 mg/l, while the concentration of the organic carbon was constant at 400 mg/l, in order to avoid carbon limitations in the bulk liquid. Maximum reduction rates of 4.8 and 4.7 g Cr(VI)/d were observed for feed Cr(VI) concentration of about 5 mg Cr(VI)/l, for the filters with the plastic support material and the gravel media, respectively. The reduction rates were significantly affected by the feed Cr(VI) concentration in both bioreactors. A dual-enzyme kinetic model was used in order to describe Cr(VI) reduction by aerobically grown mixed cultures. Model predictions were found to correspond very closely to experimental quantitative observations of Cr(VI) reduction at both pilot-scale trickling filters used.

Keywords: Hexavalent chromium; Biological reduction; Trickling filter; Plastic media; Gravel; Dual-enzyme model

1. Introduction

Chromium is one of the most widely used metals in industry (metal finishing, petroleum refining, iron and steel industries, leather tanning, inorganic chemical production, textile manufacturing and pulp producing, power plants and nuclear facilities) resulting in large quantities being discharged into the environment [1]. Cr(VI) is toxic, carcinogenic and mutagenic to animals as well as humans and is associated with decreased plant growth and changes in plant morphology. In contrast, trivalent chromium [Cr(III)] is relatively less toxic, less mobile [2] and even essential to human glucidic metabolism, contributing to the glucose tolerance factor necessary for insulin-regulated metabolism [3]. The discharge of Cr(VI) to surface water is regulated to below 0.05 mg/l by the U.S. EPA [4] and the European Union [5], while total Cr, including Cr(III), Cr(VI) and its other forms is regulated to below 2 mg/l.

The conventional remediation processes for soils and wastewater contaminated with Cr(VI) involve physical and chemical technologies. Physico-chemical treatment strategies are, however expensive and produce secondary waste streams that require further treatment. Biological reduction of Cr(VI) using indigenous microorganisms offer a new cost-effective and environmentally compatible technology [6].

A wide variety of bacteria are capable of reducing Cr(VI) in diverse locations (including natural ecosystems, industrial and municipal waste) indicating that Cr(VI)-reducing capability is widespread among bacteria [7] under aerobic, anaerobic or both conditions [8]. The processes by which the microorganisms interact with the toxic metals enabling their removal/and recovery are biosorption, bioaccumulation and enzymatic reduction [9].

The enzymatic mechanisms of bacterial reduction of Cr(VI) have been established; Cr(VI) reduction is catalyzed by either a soluble reductase or a membrane-bound protein. The soluble Cr(VI) reductase usually utilizes NADH as electron donor for necessity or for maximum activity, and Cr(VI) reduction by this soluble reductase acts as a cometabolism which does not generate biochemical energy to support cell growth. The activity of membrane-bound reductase, on the other hand, is probably respiratory chain linked, and Cr(VI) may be used as a terminal electron acceptor under anaerobic conditions [10]. Therefore, several researchers have proposed that enzymatic reduction of

* Corresponding author. Tel.: +30 26410 74117; fax: +30 26410 74172.
E-mail address: dvagenas@cc.uoi.gr (D.V. Vayenas).

0304-3894/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2006.11.017

Cr(VI) is believed to be mediated by enzymes that are not substrate-specific for Cr(VI) and that "chromate reductases" might be the serendipitous activity of enzymes with other primary physiological functions, since Cr(VI) in the environment is primarily anthropogenic [11].

Modeling of Cr(VI) reduction in biological systems has been proposed to predict the behavior of bacteria and performance of bioreactors in reducing Cr(VI). Shen and Wang [10] developed a model based on enzymatic reaction to characterize the rate and extent of microbial reduction of Cr(VI) in *Escherichia coli*. A finite reduction was proposed and incorporated into the enzymatic model to regulate the toxicity effect on cells due to the oxidizing power of Cr(VI). The above model was used to describe Cr(VI) biological reduction in a two-stage bioreactor system [12] and Cr(VI) reduction by pure bacterial cultures [13]. The previously described kinetic models were developed based on enzymatic Cr(VI) reduction by resting cells with concomitant inactivation of viable cells. In continuous-flow systems, new cells continuously replace inactivated cells and cells washed away from the reactor.

Nkhalambayausi-Chirwa and Wang [14] developed a model which simulates the effects of inhibition and cell inactivation on removal kinetics in a *Bacillus* sp. pure culture biofilm reactor system. The results show that cell removal was critical for the performance of the reactor to meet requirements of space for new viable Cr(VI) reducing cells in the biofilm, since without cell removal, the reactor may get overrun by expired cells and lose the capability to reduce Cr(VI).

Previous modeling on bacterial Cr(VI) reduction has been based on a single enzyme kinetic approach for aerobic and anaerobic Cr(VI) reduction. The Michaelis–Menten kinetic equation has been used in some other cases, while other researchers have modified it to include substrate inhibition [10].

A dual-enzyme kinetic model proposed by Viamajala et al. [7] is based on the assumption that two enzymes are responsible for Cr(VI) reduction; a fast acting but quickly deactivating, and a slow acting but stable, are responsible for reducing Cr(VI) to Cr(III) and these enzymatic reactions are simultaneous in a system with a stable cell concentration. Thus, via two parallel and independent mechanisms biological Cr(VI) reduction can be described.

At the present study, the use of mixed aerobically grown indigenous cultures, attached on the support media of pilot-scale trickling filters resulted in Cr(VI) reduction, was examined. The effect of feed Cr(VI) concentration on filter efficiency was studied even for very high Cr(VI) concentrations, up to 100 mg/l. The dual-enzyme kinetic model was used to simulate biological Cr(VI) reduction since experimental observations revealed that more than one reduction mechanism took place by aerobically grown mixed cultures.

2. Materials and methods

2.1. Media

The influent feed to the bioreactors was prepared by dissolving 1 g NH_4Cl, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.001 g $FeSO_4{\cdot}7H_2O$, 0.001 g $CaCl_2{\cdot}2H_2O$, 2.5 g $CH_3COONa{\cdot}3H_2O$ and 0.5 g K_2HPO_4 in 1.0 l of tap water.

2.2. Reagents

Stock Cr(VI) solution (500 mg/l) was prepared by dissolving 141.4 mg of 99.5% $K_2Cr_2O_7$, previously dried at 103 °C for 2 h, in Milli-Q water and diluting to 100 ml. Diphenyl carbazide solution was prepared by dissolving 250 mg of 1,5-diphenylcarbazide in 50 ml of HPLC-grade acetone and was stored in a brown bottle, for Cr(VI) determination. 1,5-Diphenylcarbazide was purchased from Fluka Chemical, Potassium dichromate was purchased from Sigma Chemical Co.

2.3. Analytical methods

During all experiments, hexavalent chromium concentration, pH, temperature, and dissolved oxygen concentration measurements were made on a daily basis. Samples were filtered through 0.45 μm—Millipore filters (GN-6 Metricel Grid 47mm, Pall Corporation). Hexavalent chromium concentration was determined by the 3500-Cr D Colorimetric method according to Standard Methods for the Examination of Water and Wastewater [15]. The cell density of liquid culture was determined as optical density at 600 nm on a Jasco V-530, spectrophotometer. The preparation of the enrichment of indigenous bacteria was described in Dermou et al. [16].

2.4. Reactor system

Two pilot-scale trickling filters were constructed for biological Cr(VI) reduction. The pilot-scale trickling filters consisted of a Plexiglas tube, 160 cm high and 9 cm i.d. This pilot-filter height is typical of a full-scale industrial filter. Since it is the loadings (hydraulic and chromium) per unit cross-sectional area that matter, no scale-up was necessary. Two different support materials were used for the experiments. The first type was hollow plastic tubes, 1.6 cm internal diameter, 3 cm length, specific surface area 500 m^2/m^3 and filter porosity 0.8. The second type was calcitic gravel, mean diameter 5.5 mm, specific surface area 1059 m^2/m^3 and filter porosity 0.4. The depth of the support media was about 143 cm in both cases. The working volumes of the reactors after several operating cycles were about 7.5 and 3.3 l, respectively. The formation of sediments caused fluctuation on the working volumes of the reactors. Chromate sediments and excess biomass were removed during the backwashing procedure from the entire volume of the reactors. After backwashing, the trickling-filters reached a new steady state within 2 h. Elemental analysis of the sediments showed that 44% of sediment was consisted of organic matter and 56% was consisted of chromate sediment, probably $Cr(OH)_3$ (Perkin-Elmer 2400 CHNS Analyzer).

At the top of the filter a fixed nozzle distributed the incoming solution evenly to the whole filter surface. The support material was not flooded for flow rates up to 3000 ml/min or hydraulic loadings up to 680 m^3/m^2 d. The filter was also equipped with an underdrain system to collect the treated water

and any biological solids that would detach from the media. Along the filter depth there were 10 sampling ports for Cr(VI) concentration measurements in the bulk liquid in order to monitor the spatial distribution (homogeneity) of the bulk liquid concentration.

The pilot-scale trickling filters were operated at SBR operating mode with recirculation. The recirculation was necessary in order to provide sufficient aeration and to obtain completely mixed flow in the bioreactors, since the formation of chromate precipitates was leading to spatial heterogeneity and consequently to insufficient exploitation of the filters. The recirculation stream that was adopted had the value of 2400 ml/min for both filters, as it was providing efficient filter wetting without flooding. Throughout all experiments water temperature was fairly constant at about $28 \pm 1\,^{\circ}C$ (ambient temperature $26\,^{\circ}C$). The pH ranged from 7.2 to 8.87 and the concentration of the dissolved oxygen in the liquid phase was physically maintained at a near constant level of 4.5 mg DO/l.

2.5. Reactors start-up

An inoculum was taken from the previous grown culture [16] and a new culture was prepared at 30 mg/l Cr(VI) in a 2 l Erlenmeyer flask, while sodium acetate (carbon source) was used in excess. Samples form this liquid culture was used to inoculate the bioreactors at the beginning of their operation.

For immobilization of the chromate-reducing bacteria, the procedure described in Dermou et al. [16] was followed. The pilot plants were kept under SBR operation mode with recirculation for several operating cycles, until constant Cr(VI) reduction rates achieved for the same inlet Cr(VI) concentration.

Before biological filters operation commenced, two experiments were performed: (a) to investigate possible physicochemical Cr(VI) reduction under the specific experimental conditions and (b) to test sorption of Cr(VI) in the mixed culture biofilm.

No abiotic physicochemical Cr(VI) removal was observed as the effluent Cr(VI) concentration reached the influent level and remained constant, even after the addition of the carbon source [16].

An initial rapid rate of Cr(VI) reduction has been observed with other microbial systems and has been attributed to biosorption [17]. To test sorption of Cr(VI) in the cells, mixed microbial cultures were washed. Two operating cycles were performed at about 5 and 30 mg Cr(VI)/l with and without sodium acetate (Fig. 1). Cr(VI) was added in the filters and no significant change in Cr(VI) concentration was observed even after 4 h of aerobic operation without sodium acetate addition. Thus, it seems likely that biosorption could not be an effective mechanism for Cr(VI) reduction in our system.

2.6. Model description

In order to describe and predict accurately biological Cr(VI) reduction the dual-enzyme kinetic model of Viamajala et al. [7] was adopted. Viamajala et al. [18] reported that *Shewanella oneidensis* MR-1 under anaerobic conditions can effectively reduce Cr(VI) and in the sequel, the same research group [7] developed

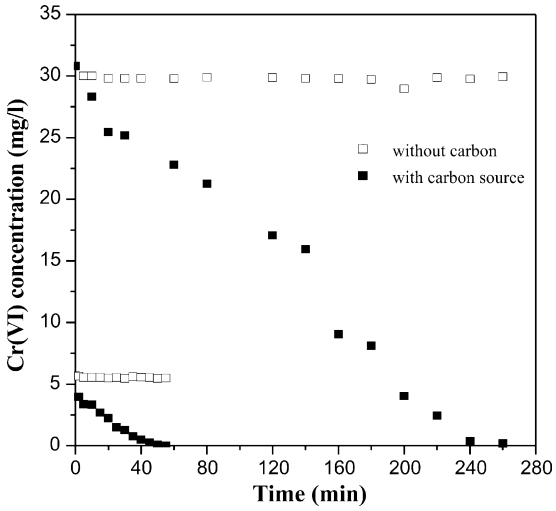

Fig. 1. Cr(VI) sorption tests under SBR operation with recirculation at feed concentrations of about 5 and 30 mg/l Cr(VI), for different filter media.

a nonlinear dual-enzyme kinetic model to simulate the multi-mechanism reduction for a batch reactor, which was developed to describe Cr(VI) reduction by *S. oneidensis* MR-1 cultures. The model is based on the assumption that two enzymes—a fast acting but quickly deactivating, and a slow acting but stable, are responsible for reducing Cr(VI) to Cr(III) and the enzymatic reactions are simultaneous in a system with a stable cell concentration.

The dual-enzyme kinetic model is based on the following reaction scheme [7]:

$$\mathrm{Cr(VI)} + \mathrm{E_d} \rightarrow \mathrm{E_d}^* + \mathrm{Cr}^* \quad (1)$$

$$\mathrm{Cr(VI)} + \mathrm{E_s} \rightarrow \mathrm{E_s} + \mathrm{Cr}^* \quad (2)$$

In the above equations, E_d represents the "deactivating enzyme" which reacts rapidly, but is converted to the inactivate form E_d^*, during reaction Cr(VI), E_s the slower "stable enzyme" that is not deactivated by Cr(VI) reduction, and Cr^* is the reduced chromium species that is produced during the enzymatic reactions.

Hossain [19] developed a graphical estimation of the dual-enzyme kinetic parameters for Cr(VI) reduction and concluded with the following equation for a batch reactor:

$$\mathrm{Cr} = \mathrm{Cr_0} - \left[\alpha t + \frac{\beta}{\gamma}(1 - \mathrm{e}^{-\gamma t})\right] \quad (3)$$

In the above equation, Cr is the Cr(VI) concentration at any time, Cr_0 the Cr(VI) concentration at time $t = 0$, α the rate constant for the stable enzyme, t the time, and β and γ are the rate constants for the deactivating enzyme. Eq. (3) can be differentiated with respect to time:

$$-\frac{\mathrm{dCr}}{\mathrm{d}t} = \alpha + \beta\,\mathrm{e}^{-\gamma t} \quad (4)$$

Eq. (4) represents the governing model equation, is nonlinear, time dependent, and "zero" order. Stable-enzyme induced reaction rate is independent of time and proceeds at a constant rate

of α. Deactivating-enzyme induced reaction rate declines exponentially with time with the highest rate β being at the beginning [19].

At the present study, two trickling filters under SBR operating mode with high recirculation rate were tested for biological Cr(VI) reduction. This operating mode (absent of inflow–outflow and high recirculation) ensured completely mixed conditions for Cr(VI) concentration in the bulk liquid, everywhere in the filters. Consequently, the reactors considered operating in batch mode. Based on our experimental observations, the formation of chromate sediments was very intense under all Cr(VI) concentrations tested (5–100 mg/l) and support media particles were always heavily coated with Cr(III) precipitates. Therefore, we assumed that Cr(VI) diffusion in the biofilm was rather rare and enzymatic extracellular reduction in the bulk liquid was the main mechanism of Cr(VI) reduction.

According to the previous assumptions the problem of mass transfer and diffusion of Cr(VI) in the filters is simplified to the problem of biological Cr(VI) reduction in a suspended growth batch reactor with the same working volume.

3. Experimental results

Aerobically grown mixed cultures were exposed to six different Cr(VI) concentrations of about 5, 10, 20, 30, 50 and 100 mg/l, while the concentration of the organic carbon was constant at 400 mg/l, in order to avoid carbon limitations in the bulk liquid. Experimental results (Figs. 2 and 3) demonstrated that there was a rapid initial Cr(VI) reduction that diminished within the first minutes of the process and seemed to be dependent of the initial Cr(VI) concentrations tested (5–100 mg/l Cr(VI)), while this reliance seems to be stronger for the smaller initial concentrations (5–20 mg/l Cr(VI)). The rapid initial rate was followed by a secondary Cr(VI) reduction rate that was slower and almost constant through the remainder of the experiments. This type

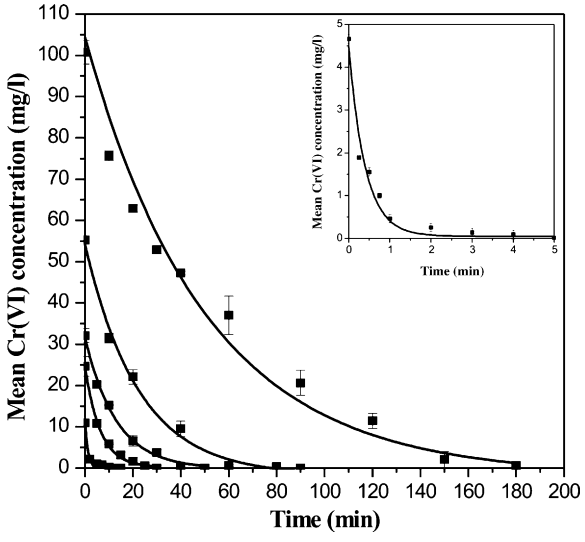

Fig. 2. Cr(VI) reduction under SBR operation with recirculation for the filter with the gravel support media at feed concentration of about 5, 10, 20, 30, 50 and 100 mg/l Cr(VI).

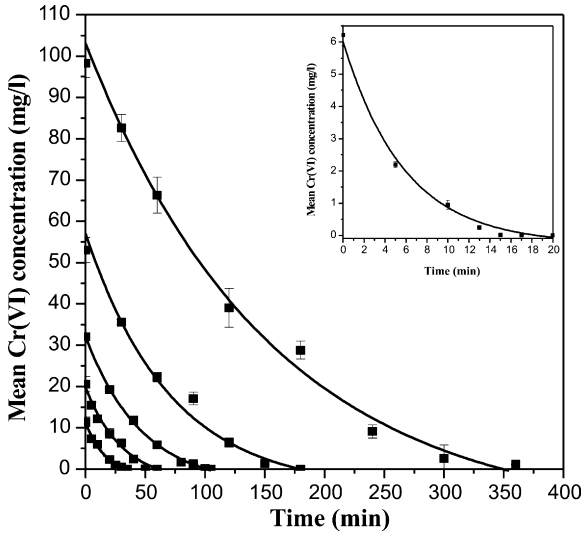

Fig. 3. Cr(VI) reduction under SBR operation with recirculation for the filter with the plastic support media at feed concentration of about 5, 10, 20, 30, 50 and 100 mg/l Cr(VI).

of reduction is in agreement with observations of Viamajala et al. [11]. At all six different concentrations tested at both pilot-plants, Cr(VI) was reduced completely by the mixed aerobically grown culture. Cr(VI) reduction profiles, as mentioned, in our studies show that the time interval for complete Cr(VI) reduction depends strongly on the initial Cr(VI) concentration (Figs. 2 and 3). The smaller the concentration tested, the smaller the time required for complete Cr(VI) reduction.

Based on the above observations we used the dual-enzyme kinetic model proposed by Viamajala et al. [7] and enhanced by Hossain [19] in order to describe Cr(VI) reduction by the aerobically grown mixed cultures. The kinetic parameters α, β and γ were estimated using the OriginPro 7.0 (nonlinear curve fitting tool), by identifying optimal values of α, β and γ using a Levenberg–Marquadt least-squares algorithm.

3.1. Experimental results and model simulations

The data points on the graphs represent average values of triplicate experiments. Kinetic parameters were estimated from each experimental data set. Model runs for the pilot-scale trickling filters are shown as the solid lines in Figs. 2 and 3.

Figs. 2 and 3 present experimental and predicted profiles of Cr(VI) reduction regarding the time required for complete Cr(VI) reduction. Six different feed Cr(VI) concentrations examined for the filter filled with gravel media and for the filter filled with plastic media, respectively. It is obvious that model fits correspond very closely to experimental observations, and that Cr(VI) reduction depends strongly on the initial concentration forced at the system for both pilot-scale trickling filters. However, the filter filled with gravel media presented complete Cr(VI) reduction under smaller time intervals. Since enzymatic Cr(VI) reduction takes place mainly in the bulk liquid, the above observation was rather expected due to the smaller working volume of the reactor compared to the equivalent time intervals for the filter filled with plastic media.

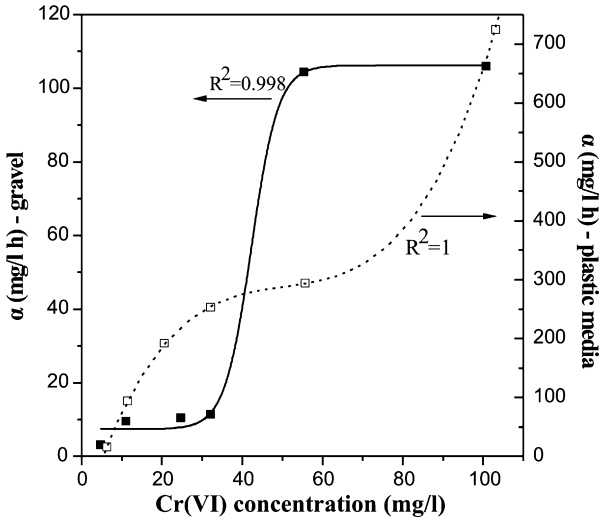

Fig. 4. Rate constant for the stable enzyme induced reaction rate (α) for the filters filled with gravel and plastic media.

Following figures (Figs. 4–6) present the values of the rate constants for the stable enzyme induced reaction rate α ($mg\,l^{-1}\,h^{-1}$), and the values of the rate constants for the deactivating enzyme β ($mg\,l^{-1}\,h^{-1}$) and γ (h^{-1}). These parameter values were computed based on the methodology outlined in the preceding section for the various Cr(VI) concentration tested for the pilot-scale trickling filters used.

The rate constant for the stable enzyme induced reaction rate, α, for the filter filled with gravel, can be described by a sigmoid equation (Fig. 4), while the rate constant for the deactivating enzyme induced reaction rate, β, can be described by a linear equation (Fig. 5). The rate constant, γ, declines exponentially with an increase of the initial Cr(VI) concentration in the trickling filter (Fig. 6).

Respectively, the rate constant for the stable enzyme induced reaction rate, α, for the filter filled with plastic media, can be described by a third order polynomial equation (Fig. 4), while the rate constant for the deactivating enzyme induced reaction rate, β, can be described by a linear equation (Fig. 5). The rate constant, γ, declines exponentially with an increase of the initial Cr(VI) concentration in the trickling filter (Fig. 6).

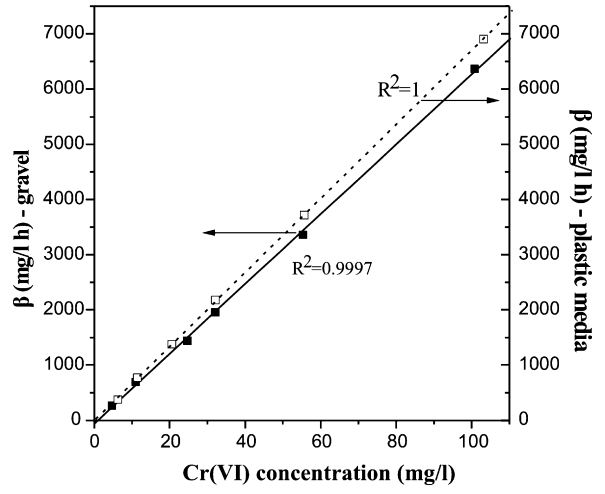

Fig. 5. Rate constant for the deactivating enzyme induced reaction rate (β) for the filters filled with gravel and plastic media.

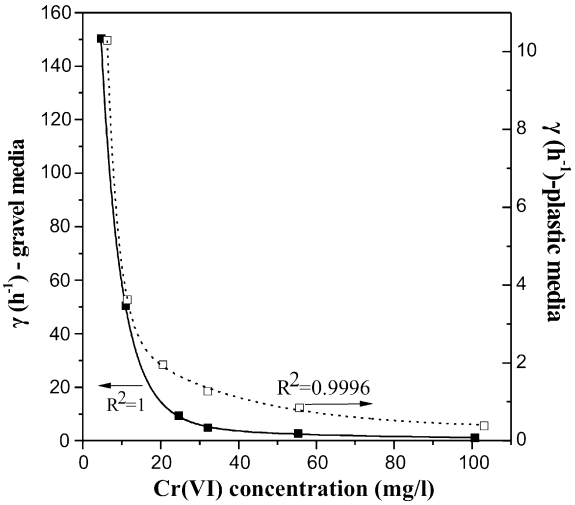

Fig. 6. Rate constant for the deactivating enzyme induced reaction rate (γ) for the filters filled with gravel and plastic media.

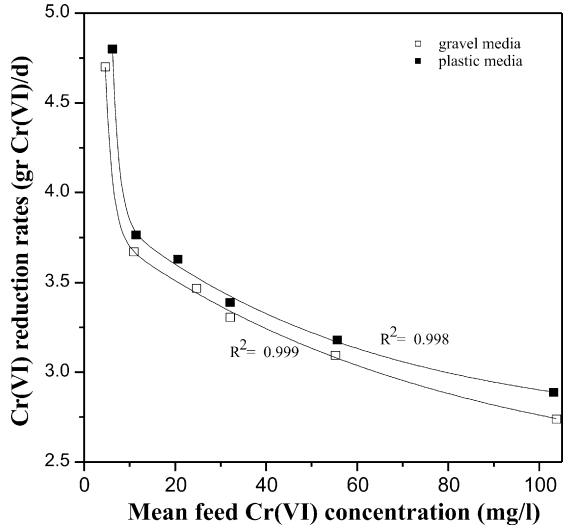

Fig. 7. Cr(VI) reduction rates under SBR operation with recirculation at various Cr(VI) concentrations for both pilot-scale trickling filers.

Fig. 7 presents Cr(VI) reduction rates obtained from SBR operation with recirculation in trickling filters operating with both gravel and plastic media.

4. Discussion

Kinetic parameters were estimated from each experimental data set for each bioreactor separately. The rate constants for the deactivating enzyme induced reaction rate incurred the same changes for both bioreactors as the feed Cr(VI) concentration increased. The rate constant β can be described by a linear expression for both pilot-scale trickling filters, while the values are similar for both bioreactors (Fig. 5). The rate constant γ declines exponentially for both pilot-scale trickling filters, while the values are greater (one order of magnitude) for the filter filled

with gravel media compared to those of the filter with plastic media (Fig. 6). The above observation led to the conclusion that the deactivating enzyme is becoming inactive faster for the filter filled with gravel media. The smaller void space between the gravels and the sediments formation restrain the working liquid volume, thus resulting in limitation of the deactivating enzyme reductive effect. This conclusion is in agreement with the experimental observations.

The rate constant for the stable induced reaction rate α increases as feed Cr(VI) concentration is raised to the value of 50 mg/l for the filter filled with gravel media. Upon this concentration value the rate constant becomes steady. It seems that the stable enzyme induced reaction rate for the gravel media presents a finite reduction capacity, while the equivalent rate constant, for the plastic media, has not reached any finite capacity for the concentrations tested (Fig. 4). The above observation results from the different features of the support materials. Plastic media offer less specific surface area but filter's porosity is increased (double than gravel). Sediment formation and deposition have a better distribution through the entire volume of the filter. The working liquid volume in which enzymatic reaction mainly takes place is greater, due to the increased void space leading to greater values obtained for the rate constant of the stable induced reaction rate, since the reaction/reduction activity remains unhindered for prolonged time intervals.

It should be noted that in previous works [7,11,18,19] where the dual-enzyme model was applied the values of the kinetic parameters were considered as mean values since experiments were performed for a very narrow range of concentrations. It is the first time that well defined experiments were performed for such a wide range of Cr(VI) feed concentrations (5–100 mg/l) and the behavior of the kinetic parameters has been described accordingly.

The type of the support media determines the working volume of the wastewater to be treated and is the surface to which the microorganisms can become attached. The use of an attached growth system provides the necessary surface for the development of biofilm structures. Biofilms provide high biomass concentration per unit volume, while bacteria can remain in the reactor for unlimited time, thus allowing the bacteria better adjustment to the environmental conditions.

Specific surface area is another parameter affected drastically by the type of the support media. Gravel offers higher specific surface area but lower working wastewater volume. Biofilm development is limited by the reduced void space between the gravels while pore clogging tends to be a crucial problem. Plastic media offer less (half than the gravel) specific surface area but the wastewater working volume is increased (double than gravel). The increased void space allows the development of very thick biofilm formations. In addition, sediment formation and deposition have a better distribution through the entire volume of the filter leading to high chromate reduction rates. Pore clogging was rather rare in the filter with the plastic media and consequently filter operation was uninterrupted for longer periods.

The use of two different support materials led to similar filter performances under SBR operation with recirculation (Fig. 7). Plastic media showed slightly better performance compared to gravel media. The use of plastic media diminishes operating disturbances during industrial applications, since the plastic media enables the growth of a thicker biofilm layer and avoids pore clogging. That makes plastic media preferable towards gravel media for industrial use.

It can been seen that the efficiency of the reduction rates diminish while increasing the initial Cr(VI) concentration at the bulk liquid. Particularly, the reduction rates for the plastic media achieved were 4.8 ± 0.02, 3.8 ± 0.009, 3.6 ± 0.02, 3.4 ± 0.03, 3.2 ± 0.02 and 2.9 ± 0.01 g Cr(VI)/d, for feed concentrations of Cr(VI) of about 5, 10, 20, 30, 50 and 100 mg/l, respectively, while for the gravel media the corresponding rates were 4.7 ± 0.02, 3.7 ± 0.02, 3.5 ± 0.06, 3.3 ± 0.04, 3 ± 0.06 and 2.7 ± 0.07 g Cr(VI)/d. However, the reduction rates that were accomplished are among the greatest ever reported in literature [13,20].

5. Conclusions

Biological Cr(VI) reduction was proved efficient with the use of pilot-scale attached growth bioreactors and indigenous bacterial population, which was grown aerobically with sodium acetate as the sole electron donor. The main conclusions from this work are:

- Experimental results clearly demonstrated that Cr(VI) reduction depends strongly on the initial Cr(VI) concentration for both support materials tested. An increase of the feed Cr(VI) concentration decreases the reduction rates of the filters.
- The operation of two pilot-scale bioreactors under SBR mode with recirculation can be described by a nonlinear dual-enzyme kinetic model.
- Experimental results indicated the presence of (a) a rapid enzymatic mechanism that is deactivating during Cr(VI) reduction, and (b) a slower enzymatic mechanism that remains active during Cr(VI) reduction, which is consistent with previous studies [7,11,18,19].
- Model fits were found to correspond very closely to experimental observations for a wide range of Cr(VI) concentrations (5–100 mg/l) under aerobic conditions for both pilot-scale trickling filters used.
- Kinetic parameters of the dual-enzyme model were estimated for a wide range of Cr(VI) concentrations tested and proved to be seriously affected from the feed concentration.
- The mathematical model applied could be an excellent tool for the prediction of Cr(VI) reduction from industrial effluents and the design of corresponding treatment plants.
- Plastic media with high porosity proved to be more efficient for industrial applications than gravel media.
- SBR operation with recirculation for the filter with plastic support media led to significant reduction rates up to 4.8 g Cr(VI)/d.

Acknowledgment

This work was supported by the Hellenic Aerospace Industry S.A.

References

[1] Y.T. Wang, H. Shen, Bacterial reduction of hexavalent chromium, J. Ind. Microbiol. 14 (1995) 159–163.

[2] K. Rama Krishna, L. Philip, Bioremediation of Cr(VI) in contaminated soils, J. Hazard. Mater. B 121 (2005) 109–117.

[3] A.M. Zayed, N. Terry, Chromium in the environment: factors affecting biological remediation, Plant Soil 259 (2003) 139–156.

[4] A. Baral, R.D. Engelken, Chromium-based regulations and greening in metal finishing industries in the USA, Environ. Sci. Policy 5 (2) (2002) 121–133.

[5] EC-Official Journal of the European Communities, L330/32, December 12, 1998.

[6] F.A.O. Camargo, B.C. Okeke, F.M. Bento, W.T. Frankenberger, Diversity of chromium-resistant bacteria isolated from soils contaminated with dichromate, Appl. Soil Ecol. 29 (2005) 193–202.

[7] S. Viamajala, B.M. Peyton, J.N. Petersen, Modeling chromate reduction in *Shewanella oneidensis* MR-1: development of a novel dual-enzyme kinetic model, Biotechnol. Bioeng. 83 (7) (2003) 790–797.

[8] A. Pal, A.K. Paul, Aerobic chromate reduction by chromate-resistant bacteria isolated from serpentine soil, Microbiol. Res. 159 (2004) 347–354.

[9] T. Srinarth, T. Verma, P.W. Ramteke, S.K. Garg, Chromium(VI) biosorption and bioaccumulation by chromate resistant bacteria, Chemosphere 48 (2002) 427–435.

[10] H. Shen, Y.T. Wang, Modeling hexavalent chromium reduction in *Escherichia coli* 33456, Biotechnol. Bioeng. 43 (1994) 293–300.

[11] S. Viamajala, B.M. Peyton, W.A. Apel, J.N. Petersen, Chromate/ nitrite interactions in *Shewanella oneidensis* MR-1: evidence for multiple hexavalent chromium [Cr(VI)] reduction mechanisms dependent on physiological growth conditions, Biotechnol. Bioeng. 78 (2002) 770–778.

[12] H. Shen, Y.T. Wang, Hexavalent chromium removal in two-stage bioreactor system, J. Environ. Eng. 21 (11) (1995) 798–804.

[13] Y. Wang, H. Shen, Modelling Cr(VI) reduction by pure bacterial cultures, Water Res. 31 (4) (1997) 727–732.

[14] E.M. Nkhalambayausi-Chirwa, Y.T. Wang, Modeling hexavalent chromium removal in a *Bacillus* sp. Fixed-film bioreactor, Biotechnol. Bioeng. 87 (7) (2004) 874–883.

[15] APHA, AWWA, WPCF, Standard Methods for the Examination of Water and Wastewater, 17th ed., American Public Health Association, American Water Works Association and Water Pollution Control Federation, Washington, DC, 1989.

[16] E. Dermou, A. Velissariou, D. Xenos, D.V. Vayenas, Biological chromium(VI) reduction using a trickling filter, J. Hazard. Mater. B 126 (2005) 78–85.

[17] S. Singh, B.N. Rai, L.C. Rai, Ni(II) and Cr(VI) sorption kinetics by *Microcystis* in a single and multimetallic system, Process Biochem. 36 (2001) 1205–1213.

[18] S. Viamajala, B.M. Peyton, W.A. Apel, J.N. Petersen, Chromate Reduction in *Shewanella oneidensis* MR-1. Is an inducible process associated with anaerobic growth, Biotechnol. Prog. 18 (2002) 290–295.

[19] Md.A. Hossain, Graphical estimation of the dual-enzyme kinetic parameters for Cr(VI) reduction, Chemosphere 63 (2006) 171–174.

[20] Y.T. Wang, C. Xiao, Factors affecting hexavalent chromium reduction in pure cultures of bacteria, Water Res. 29 (11) (1995) 2467–2474.